



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

CAHOON ET AL.

CASE NO: BB1295 US CNT

SERIAL NO: 10/690,994

GROUP ART UNIT: 1638

FILED: OCTOBER 21, 2003

EXAMINER: L. ZHENG

FOR: PLANT DIACYLGLYCEROL

ACYLTRANSFERASES

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450

Sir:

I, Keith R. Roesler, declare that:

I am a citizen of the United States and reside at 3928 Patricia Drive, Urbandale, Iowa, 50322.

I am an employee of Pioneer Hi-Bred International, Inc., ("Pioneer") a subsidiary of E.I. du Pont de Nemours and Company ("DuPont").

I received a Ph.D. in Agronomy from the University of Illinois at Urbana-Champaign. I have worked for Pioneer from July 1, 1995, to the present in the fields of biochemistry and molecular biology.

I am familiar with the subject matter of the above-identified application and supervised the performance of the experiments explained below.

The following are my remarks:

- 1. In the December 18, 2006, Non-Final Office Action related to the above-identified application, the Examiner *inter alia* rejected claims 26-40 under 35 U.S.C. § 101 because the claimed invention is allegedly not supported by either a specific and/or substantial asserted utility or a well-established utility.
- 2. Described herein are a series of experiments that establish a specific and substantial asserted utility for the claimed inventions.

Serial No. 10/690,994 Docket No. BB1295 US CNT

- 3. The basic experimental procedure is as follows. A *Saccharomyces cerevisiae* strain was created with two genes deleted: the *DGA1* gene, encoding acyl-CoA:diacylglycerol acyltransferase (DGAT), and the *LRO1* gene, encoding phospholipid:diacylglycerol acyltransferase (PDAT). In this yeast strain, DGAT genes were overexpressed using a strong constitutive promoter from the yeast phosphoglycerate kinase gene, and using uracil selection. DGAT assays were done using microsomal membrane preps.
- 4. The method of Milcamps *et al.*, 2005, "Isolation of a gene encoding a 1,2-diacylglycerol-*sn*-acetyl-CoA acetyltransferase from developing seeds of *Euonymus alatus*", J. Biol. Chem. 280:5370-5377 (attached herewith), was followed, with minor changes. *Saccharomyces cerevisiae* cultures were grown to early stationary phase in 100 ml of SC media minus uracil. Following harvest, the yeast pellets were resuspended in 4 ml of 20 mM Tris-HCl, pH 8, 10 mM MgCl₂, 1 mM EDTA, 5% glycerol, 1 mM DTT, and 0.3 M (NH₄)₂SO₄. Two ml of glass beads were added, and cells were lysed by vortexing for 5 min. The lysate was centrifuged for 15 min at 1500 g at 6 °C. The supernatant was then centrifuged at 100,000 g for 1.5 hr at 6 °C. The microsomal pellet was resuspended in 500 μl of 100 mM potassium phosphate, pH 7.2, containing 10% glycerol and frozen in liquid nitrogen prior to storage at -80 °C. Protein concentrations were determined by the method of Bradford, using the Coomassie Plus reagent (Pierce), with bovine serum albumin as standard.
- 5. DGAT assays were done for 1 min at 25 °C with 50 mM potassium phosphate pH 7.2, 10 μM 1-¹⁴C-labeled oleoyl-coenzyme A (50 mCi/mmol, Perkin Elmer), and 20 μg of microsomal protein, using endogenous diacylglycerol, in a total reaction volume of 100 μl. The reaction was started by addition of the microsomal membranes to the remainder of the reaction components. The assay was stopped and lipids were extracted with 2 ml of hexane:isopropanol (3:2) (Hara and Radin, 1978, "Lipid extraction of tissues with a low-toxicity solvent", Anal. Biochem. 90:420-426) containing 4 μl of unlabeled triacylglycerol (triolein, Sigma). Following vortexing for 10 sec, the phases were separated with 1 ml of 500 mM sodium sulfate and vortexing was again done for 10 sec. After 10 min, the upper phase was transferred to another tube and dried with nitrogen gas. The lipid was resolubilized in a small volume of hexane (approximately 100 to

Serial No. 10/690,994 Docket No. BB1295 US CNT

150 µl) and applied to K6 silica TLC plates, which were developed in 80:20:1 (v/v/v) hexane:diethylether:acetic acid. Triacylglycerol was visualized and marked by staining in iodine vapor. After the stain faded, the triacylglycerol was scraped, and radioactivity was determined by liquid scintillation counting. The following results were obtained.

Construct		ivity (pmol ¹⁴ C-l al protein)	labeled TAG pro	oduced/min/mg
	Rep1	Rep2	Rep3	Mean ± SD
Soybean DGAT	840	887	876	868 ± 25
Wheat DGAT	518	529	515	521 ± 7
Vector control	33	14	17	21 ± 10

- 6. The soybean DGAT protein-coding region was obtained by PCR using clone sr1.pk0098.a8 as a template. This sequence was used to create the yeast expression vector, PHP32069 (Appendix A). The sequence of the soybean DGAT-coding region, "PHP32069 Soy DGAT1 CDS", was found to have a one nucleotide difference with the corresponding DGAT-coding region of SEQ ID NO:15, "BB1295 SEQ-15 CDS". An alignment of the two DGAT-coding sequences is presented in Appendices B1-B4. The soybean DGAT sequence in PHP32069 has an adenine (A) at position 939 of the DGAT-coding region, while the corresponding nucleotide in SEQ ID NO:15 is cytosine (C). The cDNA insert in clone sr1.pk0098.a8 was re-sequenced, and the sequence of the DGATcoding region in sr1.pk0098.a8 was identical to the sequence of the PCR-derived DGAT-coding region in PHP32069. The single nucleotide difference in SEQ ID NO:15, also obtained by sequencing clone sr1.pk0098.a8, is presumably due to a sequencing error. This single nucleotide difference results in a one amino acid difference between the two corresponding amino acid sequences (Appendix C). The DGAT protein encoded by PHP32069 has a glutamic acid residue (E) at position 313, while the corresponding residue in SEQ ID NO:16 is aspartic acid (D).
- 7. The wheat DGAT DNA was obtained by a combination of PCR using clone wr1.pk0119.b6:fis as a template and a synthetic gene fragment to complete the coding region. This DGAT-coding sequence was used to create the yeast expression vector, PHP32068 (Appendix D). The sequence of this wheat DGAT-

Serial No. 10/690,994 Docket No. BB1295 US CNT

coding region, "PHP32068 Wheat DGAT1-2 CDS", was found to have two single nucleotide differences with the corresponding DGAT-coding region of SEQ ID NO:21, "BB1295 SEQ-21 CDS". An alignment of the two DGAT-coding sequences is presented in Appendices E1-E4. The wheat DGAT nucleotide sequence of PHP32068 has a guanosine (G) at position 303 and a thymidine (T) at position 393; the sequence of SEQ ID NO:21 has a thymidine (T) and cytosine (C) at these two positions, respectively. These two nucleotide differences are "silent", i.e., the amino acid sequence encoded by PHP32068 is identical to that of SEQ ID NO:22 (Appendix F).

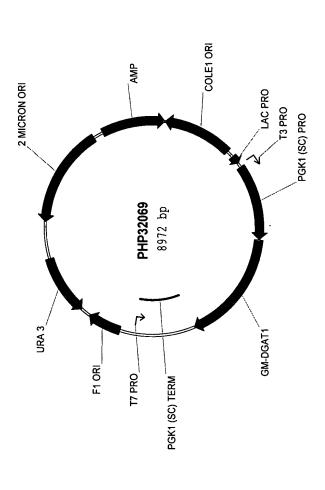
- 8. Sequence alignments were performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal V method of alignment with the default parameters
- 9. The vector control contained no DGAT gene.
- 10. As shown in the table above, both the soybean DGAT protein encoded by PHP32069 and the wheat DGAT protein encoded by PHP32068 have significant DGAT activity.
- 11. I believe that the experiments conducted thus establish a specific and substantial utility for the claimed inventions.
- 12. I declare that all statements made herein are either based on my own knowledge and are true, or if based on information and belief are believed to be true. I also declare that all statements were made with knowledge that willful false statements, and the like, are punishable by either fine, or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and any such willful false statements may jeopardize the validity of either the patent application. or any patent issuing thereon.

By: Keith R. Roesler

Dated: 5-14-07



APPENDIX A YEAST EXPRESSION VECTOR FOR SOYBEAN DGAT



APPENDIX B1 SOYBEAN DGAT NUCLEOTIDE SEQUENCE ALIGNMENT

Consensus #1	BB1295 SEQ-15 CDS.seq PHP32069 Soy DGAT1 CDS.seq	Consensus #1	BB1295 SEQ-15 CDS.seq PHP32069 Soy DGAT1 CDS.seq	Consensus #1	BB1295 SEQ-15 CDS.seq PHP32069 Soy DGAT1 CDS.seq	Consensus #1	BB1295 SEQ-15 CDS.seq PHP32069 Soy DGAT1 CDS.seq	Consensus #1	BB1295 SEQ-15 CDS.seq PHP32069 Soy DGAT1 CDS.seq	Consensus #1	BB1295 SEQ-15 CDS.seq PHP32069 Soy DGAT1 CDS.seq	Consensus #1	BB1295 SEQ-15 CDS.seq PHP32069 Soy DGAT1 CDS.seq	Consensus #1	BB1295 SEQ-15 CDS.seq PHP32069 Soy DGAT1 CDS.seq
ACCACTC	A A C C A C T C A A C C A C T C	TTCAAT	TTCAATT	GGATICT	GGATTC	CCCAACA	200 C C C A A C A C C C A A C A	GCCTACC	250 G C C T A C C	CAGCTCC	300 C A G C T C C C A G C T C C	GTATAGT 350	GTATAG	ATGAAGT	400 A T G A A G T A T G A A G T
TGCTCTCA	1 G C 1 C 1 C 2	0 1 0 0 0 0 0 0		TIGGCCAA	TTGGCCAATTGGCCAA	CGTCAATT	190 CGTCAATT CGTCAATT	TCAAATTC	Z40 TCAAATTC TCAAATTC	AGTCCGCT	290 A G T C C G C T A G T C C G C T	CAACCTTT	CAACCTTT CAACCTTT	AGAATTTA	390 A G A A T T T A A G A A T T T A
GTAGCCAC	G T A G C C A C G T A G C C A C	C T C C A C C G	C T C C A C C G C T C C A C C G	GT GAT GAC	GT G A T G A C	0 N C G C G C	180 G A C G C G C G A C G C G C	0 1 C C G 1 C C	230 C T C G T C C	TGAAGGAA	280 T G A A G G A A T G A A G G A A	330	G G C C T C T T	CATCATTG	380 CATCATTG CATCATTG
TGAAAGT	T G A A A G T T G A A A G T	0 K C C C A C	0 V 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	AGTICCG	A G T T C C G A G T T C C G	CAGCGAC	C A G C G A C	CTGATTT	C T G A T T T C T G A T T T	CGCAAAG	CGCAAAG	TCACGCG	T C A C G C G T C A C G C G	GCCGACT	G C C G A C T G C C G A C T
GATGAGCC	G A T G A G C C G A T G A G C C	CCGTCCCT		CCACCGAC	C C A C C G A C	TCCATCAA	TCCATCAA	ACAAGACA	220 A C A A G A C A A C A A G A C A	CCGCTCAC	27C C C C C T C A C C C C C T C A C	CGTCAGAG	CGTCAGAG	TGTGAATA	37. TGTGAATA TGTGAATA
GATTICCO	GATTTCC	T G C G C C G	ს ს ს ს	GAGACAAG	G A G A C A A G	CGACGACT	160 C G A C G A C 7 C G A C G A C 7	ACGAAAA	210 A C G A A A A A A A A A A A A A A A A A	υ υ	260 T C C G T C C C	TATTTCC 310	TATTTCC	TIGITIGC	360 T T G T T G C 7
ATGGC	1 ATGGC	TTCCC	51 T T C C C 51 T T C C C	E 0 0 0	101 CGCCT 101 CGCCT	0 6 6	151 G G T T C 151 G G T T C	GCAAA	201 G C A A A 201 G C A A A	F 0 0 F 0	251 GTCCT 251 GTCCT	GACAC	301 G A C A C 301 G A C A C	AGTCC	351 A G T C C 351 A G T C C

APPENDIX B2 SOYBEAN DGAT NUCLEOTIDE SEQUENCE ALIGNMENT

Consensus #1	BB1295 SEQ-15 CDS.seq	rhrizung soy DGAII CDS.3869 Consensus #1		BB1295 SEQ-15 CDS.seq PHP32069 Soy DGAT1 CDS.seq	Consensus #1		BB1295 SEQ-15 CDS.seq PHP32069 Soy DGAT1 CDS.seq	Consensus #1		BB1295 SEQ-15 CDS.seq PHP32069 Soy DGAT1 CDS.seq	Consensus #1		BB1295 SEQ-15 CDS.seq PHP32069 Soy DGAT1 CDS.seq	Consensus #1		BB1295 SEQ-15 CDS.seq PHP32069 Soy DGAT1 CDS.seq	Consensus #1		BB1295 SEQ-15 CDS.seq PHP32069 Soy DGAT1 CDS.seq	Consensus #1		BB1295 SEQ-15 CDS.seq PHP32069 Soy DGAT1 CDS.seq
ر 3	450 A		200	υυ	U	220	ט ט	٧	009	4 4	H	650	e e	Æ	-%-	4 4	4	750	4 4	o	- @-	ა ა
A G	V 0			o o	C		4 4 U U	υ υ		U U	r L		9 9	Ā		4 4	U U		ပပ	۷ 9		∢ ∢ ບ ບ
9	0				υ		υυ	H		H F	υ		υυ	Ü	ļ	טט	4	1	4 4	< 4		4 4
H	€ 6	- F		H H	ď		44	∢		4 4	4		« «	~		4 4	Æ		4 A	×	ŀ	4 A
1	₽ 6			H H	Ą		4 4	H		H H	U		ပပ	υ		ပပ	ט		ט ט	U	l	υυ
د د	V .			o o	U		ပ ပ ပ	T.		S	H		0 1	T G		0 0	٧.		4 4 0 0	H		H H
ī	E E				υ υ		00	H		11	r E		11	A		A A	o o		99	CT		C C
U	υ (ם נ			U		υυ	н		₽₽	ပ		טט	H		₽ ₽	U		טט	Ü		ບບ
⋖	- \$- ⋖ <	< ⊷	-8-	H H	4	-540	4 4	Ħ	590	∺ ₽	Ģ	640	ს ს	H	-69	⊢ ⊢	~	-충-	4 4	⋖	790	4 4
₹	< <			A A	T		H	Ü		υυ	×		4 A	C	-	υυ	4	l `	4 4	∢		« «
c A	4 A			99	TA		T A	ر د		00	T C		S	G T		5 5	A		A A	E D		0 U
į.	E + E			טט	Ü		99	ı.		F F	4		4 4	ī		H H	ď		טט	υ		υυ
E	F+ E			H H	E		H H	н		₽ ₽	H		H H	Ů		ს ს	H		T T	4		A A
U	ט ט			ტ ტ	ប		ს ს	υ		υu	ဗ		ს ს	b		ს ს	H			⋖		A A
*	4 *				٧		4 4	¥		A A	H		H H	H		H F4	U		ט ט	U		υυ
T	H €			00	GA		A A G G	ν V		V V	£	ŀ	++	AT		4 4	Ŧ		T A	T A		T A T A
E+	430		480	H H	ט	530	9 9	Ē	580	F F	H	-၉-	H H	A	089	4 A	E	_ _≈ _	e e	H	780	E E
υ	7 0	ט פ	4	ပပ	υ	2	ပပ	υ	2	ပပ	υ	9	ပပ	A	9	4 4	⋖.	1	4 4	υ	7	ပပ
U	0 (H H	K		4 4	υ		υu	U	ŀ	ဖဖ	Ą		< <	~		4 4	υ		υυ
T T					C A		V V	Ŧ		A F	r L		E E	T T		11	~		4 A	Ą		A T T
7 1	F 6			υυ	4		4 4				₽	İ	E E	G J		טט	υ		υυ	ď.		T A
E	€ 6			e e	υ		ပပ	⋖		4 4	E+		F F	ß		ს ს	~		4 4	U		υυ
υ	υu			ს ს	O		დ დ	υ		ပပ	⋖		~ ~	Ŧ		⊢ ⊢	E+		₽₽	⋖		VΥ
9	ט ט			H H	O		დ დ	e		t t	o.		ს ს	A		< <	E		₽₽	G		ს ს
D E	-23-1-1			9 9	T T	٥.	TT	A	٥.	4 4	r L		0 G	GT	_و_	0 U	A C	L _o _	ν ν	T G	L _o _	טט
Ü	420		470		ט	520	00	H	570	H H	F	62	H H	ī	679	F F	U	22	7 7 U U	۲ ۲	770	A A
H	₽ €	ט ו-		o o	∢		A A	Æ		4 4	ָט		ს ს	Ŀ		e e	U		ს ს	υ		ပပ
٧.	< *			⊢ ⊬	Ą	i	4 4	EH		₽₽	O		ပ ပ	O		ט ט	¥	l	4 4	⋖		A A
A A	V 4			* * U U	A		9 y	۷ ک		4 K	4		4 4	G T		9 9	G		טט	⋖		₹ ₹ ∪ ∪
۲ ک	\ \bar{\chi}{\chi}			- F	9		5 5	EH.		ь н	r U		00	Ŀ		H H	G A	Ì	که که دی دی	T G		
H	⊢ €			H H	U		ს ს	E		₽ ₽	υ		υυ	1		₽₽	H		H H	υ		υυ
<	< <			H H	H		₽ €	υ		ပပ	H		₽ ₽	υ		ပပ	¥		< <	E٠		₽₽
b	، ما			H	ט	٦	ს ს	4		4 4	F		H H	Ħ	٦	₽ ₽	Þ	٦	₽ ₽	υ	_	ပပ
T T	1 1 1	ט ד	460	o o	T A	510	T A	G T	260	9 9	T.	61(T F	T T	99	11	GA	710	V V	T	760	T A
S	0 0	ט פ		υυ	۸.		4 4	E+		E E	T			1	ŀ	E E	ı.		5 F	4		4 4
v	ט נ	ט פ		ပပ	£		e e	EH		₽ ₽	ပ		ဖဖ	b		טט	K	}	e e	v		ს ს
H	₽ 6			ს ს	H		₽ ₽	ၒ		ပ ပ	æ		4 4	H		H H	T		H H	U		υυ
€+	F+ 6			00	F		H H	۲		E F	۲		e e	Ö		υυ	U		υυ	H		H #H
o o	0 1			00	υ υ		υ υ υ υ	T D		0 U	T		11	T G	İ	9 9	A A		4 A 4 A	2		ပ ၁ ၁
F	€+ €			< <	r		ບ ບ	H		H H	E	Ì	H H	A	l	4 4	V V		4 A	ı		H H
*	< -			ს ს	H		H #	F		₽ ₽	ŋ		ပ ပ	K		4 4	υ		υυ	υ		ပပ
	401	10.7		451 451			501 501			551 551			601			651 651			701 701			751 751

APPENDIX B3 SOYBEAN DGAT NUCLEOTIDE SEQUENCE ALIGNMENT

Consensus #1	BB1295 SEQ-15 CDS.seq PHP32069 Soy DGAT1 CDS.seq	Consensus #1	BB1295 SEQ-15 CDS.seq PHP32069 Soy DGAT1 CDS.seq	Consensus #1	BB1295 SEQ-15 CDS.seq PHP32069 Soy DGAT1 CDS.seq	Consensus #1	BB1295 SEQ-15 CDS.seq PHP32069 Soy DGAT1 CDS.seq	Consensus #1	;	BB1295 SEQ-15 CDS.seq PHP32069 Soy DGAT1 CDS.seq	Consensus #1	BB1295 SEQ-15 CDS.seq PHP32069 Soy DGAT1 CDS.seq	Consensus #1	BB1295 SEQ-15 CDS.seq PHP32069 Soy DGAT1 CDS.seq	Consensus #1	,	BB1295 SEQ-15 CDS.seq PHP32069 Soy DGAT1 CDS.seq
CTTGGCATATTTCCTGGTTGCCCCTACATTATGTTACCAAGCTATC	801 CTTGGCATATTCCTGGTTGCCCTACATTATGTTACCAGCCAAGCTATTCC	CTC G C A C A C C T T A T A T T C G A A A G G G T T G G T T G T T T C G C C A A C T T G T C A A G	0 0 0 0 1 1 1	CTGATAATATACAGAGTTATGGGATTTATAATAGA.CAATAATAA 910 920 930 940 550	901 CTGATAATATTACAGGAGTTATGGGATTTATAATAGACCAATATAAA 901 CTGATAATTTACAGGAGTTATGGGGATTTAATAATAGA <mark>I</mark> SCAATATTAA	TCCCATAGTACAAATTCACAGCATCCTCTAAGGGAAACCTTCTTACG	951 TCCCATAGTACATATTCACAGCATCCTCTCAAGGGAAACCTTCTTACG 951 TCCCATAGTACAAAATTCACAGCATCCTCTCAAGGGAAACCTTCTTACG	C C A C C G A G A G T T C T G A A G C T T T C T G T T C T G T G G C T C	1010 1020 1030 1040	1001 ССАСС GAGAGTT CTGAAGCTTT CTGTT CCAAATTATATGT GTGGCTC 1001 ССАСС GAGAGTT CTGAAGCTTT CTGTTC CAAATTATATGTGTGGCTC	T G C A T G T T T T T G C T T T T C C C T T T G G T T A A T A T C T G C C A G A G C T	1051 TGCATGTTCTATTGCTTTTTGCACTTTAAATATCTGGCAGAGT 1051 TGCATGTTCTATTGCTTTTTGGTTAAATATCTGGCAGAGT 1052 TGCATGTTTTTCCACCTTTGGTTAAATATCTGGCAGAGT	TCTTCGATTTGGTGATCGTGAATTCTACAAGGATTGGTGGAATGCCAAAA	1150 1150 1150 1150 1150 1150 1150 1150	CTGTCGAAGATTATTGGAGGATGTGGAATATGCCTGTTCACAAATGGAT		1151 CTGTCGAAGATTATTGGAGGATGTGGAATATGCCCTGTTCACAAATGGATG 1151 CTGTCGAAGATTATTGGAGGATGTGGAATATGCCCTGTTCACAAATGGATG
	_ ~																

APPENDIX B4 SOYBEAN DGAT NUCLEOTIDE SEQUENCE ALIGNMENT

Consensus #1 BB1295 SEQ-15 CDS.seq PHP32069 Soy DGAT1 CDS.seq	Consensus #1 BB1295 SEQ-15 CDS.seq PHP32069 Soy DGAT1 CDS.seq	Consensus #1 BB1295 SEQ-15 CDS.seq PHP32069 Soy DGAT1 CDS.seq	Consensus #1 BB1295 SEQ-15 CDS.seq PHP32069 Soy DGAT1 CDS.seq	Consensus #1 BB1295 SEQ-15 CDS.seq PHP22069 Soy DGAT1 CDS.seq Consensus #1	BB1295 SEQ PHP32069 3	BB1295 SEQ-15 CDS.seq PHP32069 Soy DGAT1 CDS.seq
ATCCGCCACCTATATTTCCATGTTTAAGGCACGGTCTACCAAAGGCTGCGCTGCCCCCCAAAGGCTGCCCCACAAAGGCTGCCCACAAAGGCTGCCCACAAAGGCTGCCCACAAAGGCTGCCCACGCTGCCCCAAAAGGCTGCCCCCAAAAGGCTGCCCACAAAGGCTGCCCCAAAAGGCTGCCCAAAAGGCTGCC	TGCTCTTTTAATTGCCTTCCTGGTTTCTGCTTTATTCCATGAGCTGTGCA 1250 1260 1270 1280 1390 1350 1350 1251 1361 1351 1361 1351 1361 1351 1361 136	TTGCTGTTCCTTGCCACATATTCAAGTTGTGGGCTTTCGGTGGAATTATG 130 130 130 130 130 130 130 130 130 130	TTTCAGGTTCCTTTGATCACTAATTATCTGCAAAATTATCAG 1350 1351 1351 1352 1351 1352 1353 1353 1353	AAACTCAATGGTTGGAAATATGATTTTTGGTTCATATTCAGTTCCTTG 1450	1460 1470 1480 1490 1490 1490 1490 1490 1490 1490 149	1501 GGCAAACTTGAC 1501 GGCAAACTTGAC

Consensus 'Consensus #1': When all match the residue of BB1295 SEQ-15 CDS.seq show the residue of BB1295 SEQ-15 CDS.seq, otherwise show '.'.

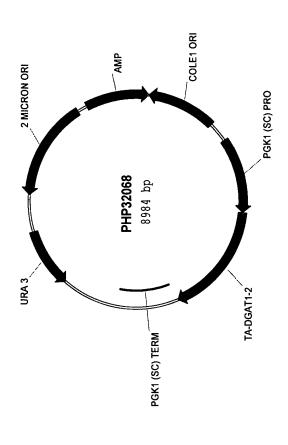
Decoration 'Decoration #1': Box residues that differ from BB1295 SEQ-15 CDS.seg.

APPENDIX C SOYBEAN DGAT AMINO ACID SEQUENCE ALIGNMENT

	MAISDEPESVATALNHSSLRRRPSATSTAGLFNSPETTTO 10 10 10 10 10 10 10 10 10 10 10 10 10	D S S G D D L A K D S G S D D S I N S D D 40 5 60 50 50 60 60 60 60 60 60 60 60 60 60 60 60 60	Consensus #1
	MAISDEPES VATALNHSSLRRRPSATSTAGLFNSPETTTD MAISDEPES VATALNHSSLRRRPSATSTAGLFNSPETTTD	D S S G D D L A K D S G S D D S I N S D D D S S S G D D L A K D S G S D D S I N S D D	BB1295 USCNT SEQ-16.pro PHP32069 Soy DGAT1.pro
	AAVNSQQQNERQDTDFSVLKFAYRPSVPAHRKVKESPLSS	S D T I F R Q S H A G L F N L C I V V L V 100 110 120	Consensus #1
61	AAVNSQQQNE KQDTDPSVLKFAYRPSVPAAAVNSQQQNE KQDTDFSVLKFAYRPSVPA	S D T I F R Q S H A G L F N L C I V V L V S D T I F R Q S H A G L F N L C I V V L V	BB1295 USCNT SEQ-16.pro PHP32069 SOY DGAT1.pro
	L M K Y G W L I K S G F W F S S K S L	SLVVEPFAAFIVEKLAQRKCI	Consensus #1
121	130 140 150 150 160 150 160 150 160 150 160 170 170 170 170 170 170 170 170 170 17	160 170 170 180 S L V V P P F A A F I V E K L A Q R K C I S L V V F P F A A F I V E K L A Q R K C I	BB1295 USCNT SEQ-16.pro PHP32069 Soy DGAT1.pro
	PEPVVVLHIIITSTSLFYPVLVILRCDSAFVSGVTLMLF	FSCVVWLKLVSYAHTNYDMRA	Consensus #1
181	THILLISTS LEYPULVIERCDS A PUSCUTLE LEVEL OF LEWIL	F S C V W L K L V S Y A H T N Y D M R A F S C V V W L K L V S Y A H T N Y D M R A	BB1295 USCNT SEQ-16.pro PHP32069 Soy DGAT1.pro
	LIKLVEKGEALLDILNMDYPYNVSFKSLAYFLVAPTLCYQ	QPSYPRTPYIRKGWLFROLVK	Consensus #1
241	LTKLVEKGEALLDTLNMDYPYNVSFKSLAYFLCY LTKLVEKGEALLDTLNMDYPYNVSFKSLAYFLVAPTLCY	O P S Y P R T P Y I R K G W L F R Q L V K Q P S Y P R T P Y I R K G W L F R Q L V K	BB1295 USCNT SEQ-16.pro PHP32069 Soy DGAT1.pro
	LIIFTGVMGFII. QYINPIVQN SQHPLKGNLLYATERVLK	KLSVPNLYVWLCMFYCFFHLW	Consensus #1
301	310 LIIFTGVMGFIIDQYINPIVQNSQHPLKGNLLYATERVL LIIFTGVMGFIIEGYINPIVQNSQHPLKGNLLYATERVL	340 350 350 360 360 X L S V P N L Y V W L C M F Y C F F H L M K L S V P N L Y V W L C M F Y C F F H L M	BB1295 USCNT SEQ-16.pro PHP32069 Soy DGAT1.pro
	INILAELLRFGDREFYKDWWNAKTVEDYRRWNMPVHKWM	MIRHLYFPCLRHGLPKAAALL	Consensus #1
361	370 380 390 LNILAELLRFGDREFYKDWWNAKTVEDYWRWWNMPVHKW LNILAELLRFGDREFYKDWWNAKTVEDYWRWWNMPVHKW	000 410 420 A A A A A L L M I R H L Y F P C L R H G L P K A A A L L L A I R H L Y F P C L R H G L P K A A A L L	BB1295 USCNT SEQ-16.pro PHP32069 Soy DGAT1.pro
	IAFLVSALFHELCIAVPCHIFKLWAFGGIMFQVPLVLITN	NYLQNKFRNSMVGNMIFWFIF	Consensus #1
421	430 440 450 1 A F E L C I A V P C H I F K L W A F G G I M F Q V P L V L I T I A F L V S A L F H E L C I A V P C H I F K L W A F G G I M P Q V P L V L I T	460 TNYLQNKFRNSMVGNMIFWFIF BB12 TNYLQNKFRNSMVGNMIFWFIF PHP3	BB1295 USCNT SEQ-16.pro PHP32069 Soy DGAT1.pro
	SILGOPMCVLLYYHDLMNRKGKLD	Cons	Consensus #1
481	490 500 11 SILGQPMCVLLYYHDLMNRKGKLD 11 SILGQPMCVLLYYHDLMNRKGKLD	BB12 PHP3	BB1295 USCNT SEQ-16.pro PHP32069 SOY DGAT1.pro
onse	Consensus 'Consensus #1': When all match the residue of BB1295 USCNT SEQ-16.pro show the residue of BB1295 USCNT SEQ-16.pro, otherwise show	f BB1295 USCNT SEQ-16.pro, otherwise show '.'.	

Decoration 'Decoration #1': Box residues that differ from BB1295 USCNT SEQ-16.pro.

APPENDIX D YEAST EXPRESSION VECTOR FOR WHEAT DGAT



APPENDIX E1 WHEAT DGAT NUCLEOTIDE SEQUENCE ALIGNMENT

Consensus #1	BB1295 SEQ-21 CDS.seq PHP32068 Wheat DGAT1-2 CDS.seq	Consensus #1	BB1295 SEQ-21 CDS.seq PHP32068 Wheat DGAT1-2 CDS.seq	Consensus #1	BB1295 SEQ-21 CDS.seq PHP32068 Wheat DGAI1-2 CDS.seq	Consensus #1	BB1295 SEQ-21 CDS.seq PHP32068 Wheat DGAT1-2 CDS.seq	Consensus #1	BB1295 SEQ-21 CDS.seq PHP32068 Wheat DGAT1-2 CDS.seq	Consensus #1	BB1295 SEQ-21 CDS.seq PHP32068 Wheat DGAT1-2 CDS.seq	Consensus #1	BB1295 SEQ-21 CDS.seq PHP32068 Wheat DGAT1-2 CDS.seq	Consensus #1	BB1295 SEQ-21 CDS.seq PHP32068 Wheat DGAT1-2 CDS.seq
ATGTCAAAAGGGAACCCAGACCTCCCGGGCAGCTTCCTCCTTC 10	ATGTCAAAA GGGAACCCAGACCGGCACCTCCCGGCAGCTTCCTCCTTC	CCACGGGGGCGCAACCCAAAACGCGGGGGGGGGGGAACGTTCCGGAAGGGAAGGGAAGGGAAGGGAAGGGAAGGGAAGGGAAGGGAAGGGG	ссъсбесесестве в в в в ссервения в в ссесесе в в в ссесе в в в ссе в в в в	ACCTCCCTCAGTTCCACCCATGGCCCCGCCCGTCGTGGCCGCTGCC		л с в в т с в с в в с с с с с с с с с с с	160 170 200 A C G A C G A C G A C C C C C C C C C C	сетсса с с в с в с с с с с с с д т с с а с с с с с с с д с с с с д с с д с с д с с д с с д с с д с с д с с д с	210 220 230 240 250 6 G C C C C A C C C C C A C C C C C A C C C A C C C A C C C A C C C A C C C A C C C A C C C A C C C A C C C A C C C C A C C C C A C C C C A C C C C A C C C C A C C C C A C C C C A C C C C C A C C C C C A C	C C C T G C T A C C G G C G C G C C C C C C C C C C C	260 270 280 280 290 300 CCCTGCTACCGGGGGGAGAGCCGGGGTCAAGGAGCCGGGGGGGG	CT. AGCTCCGACGCCATCTTCCGACAGAGCCATGCAGGTCTTCTGAATCT	C C G A C A G A G C C A T G C A G G T C T T C T G A A T C T C C G A C G A C C A T C C A G G T C T T C T G A A T C T	атосаттеттететельные саблеваса беле в сетелтат. Сабавает	TGCATTGTTGTTGCAGTGAACAGCAGGTCATTATCGAGAACT
			51 51		101		151 151		201		251 251		301		351 351

APPENDIX E2 WHEAT DGAT NUCLEOTIDE SEQUENCE ALIGNMENT

Consensus #1	BB1295 SEQ-21 CDS.seq PHP32068 Wheat DGAT1-2 CDS.seq	Consensus #1	BB1295 SEQ-21 CDS.seq PHP32068 Wheat DGAT1-2 CDS.seq	Consensus #1	BB1295 SEQ-21 CDS.seq PHP32068 Wheat DGAT1-2 CDS.seq	Consensus #1	BB1295 SEQ-21 CDS.seq PHP32068 Wheat DGAT1-2 CDS.seq	Consensus #1	BB1295 SEQ-21 CDS.seq PHP32068 Wheat DGAT1-2 CDS.seq	Consensus #1	BB1295 SEQ-21 CDS.seq PHP32068 Wheat DGAT1-2 CDS.seq		BB1295 SEQ-21 CDS.seq PHP32068 Wheat DGAT1-2 CDS.seq	BB1295 SEQ-21 CDS.seq PHP32068 Wheat DGAT1-2 CDS.seq
TAATGAAGTATGGCCTATTAATAAGAGCTGGGTTTTGGTTTAGTGCAAGA	450 460 470 470 470 470 470 470 470 470 470 47	TCGCTGGGAGATTGGCCACTTCTGATGTGCTGCCTCACTTTACCCATTTT 460 470 480 500	ATT 6 G C C A C TT C T G A T G T G C T G C C T C A C T T T A C C C A A T T T A C C C A C T T T A C C C A T T T A	CCCACTTGCTGTCTTATGACCGAAGTGGGGTCAAAGAAAG	СССАСТТ 6 СТ 6 СТ СТ САТ 6 АСС 6 А 6 А 6 А 6 СТ 6 6 С Т СААА 6 СТ САТ САТ СТ СТ САТ СТ СТ САТ СТ СТ САТ СТ	CTCCATATAATTATACAACCACTGTCCT	560 570 580 590 600 600 600 600 600 600 600 600 600 6	GAATCAGCAGTATTATCTG	ATCTATCCGTTGTTGTTGTTCTTAAGTGTAATCAGCAGTATTATCTGGAATCTAGCAGTATTATCTGGAATCTAGCAATCAGCAGTATTATCTGGAATCTAGGAATCTGG	ATTIGICTLAATGTTCATTGCAAGCATTACTTGGTTGAAGCTTGTCTCTT	ANTIGICITAAIGIICAIIGCAAGCAIIACIIGGIIGAAGCIIGII AIIIGIGIIAAAIGIICAIIGCAAGCAIIACIIGGIIGAAGCIIGII	TGCTCATACAAATTATGATATAAGGATATTGTCCCAAAGTATTGAAAA		GTGCTACACATGTCTATCGATGAGGAAACATTAAAGGCCCAA
•	401	•	451 451	•	501 5	•	551 551	•	601 601	•	651 651		701	751 751

APPENDIX E3 WHEAT DGAT NUCLEOTIDE SEQUENCE ALIGNMENT

		CDS.seq			CDS.seq			CDS.seq			CDS.seq			CDS.seq			CDS.seq			CDS.seq			CDS.seq
		BB1295 SEQ-21 CDS.seq PHP32068 Wheat DGAT1-2 CDS.seq			BB1295 SEQ-21 CDS.seq PHP32068 Wheat DGAT1-2 CDS.seq			BB1295 SEQ-21 CDS.seq PHP32068 Wheat DGAT1-2 CDS.seq			BB1295 SEQ-21 CDS.seq PHP32068 Wheat DGAT1-2 CDS.seq			BB1295 SEQ-21 CDS.seq PHP32068 Wheat DGAT1-2 CDS.seq			BB1295 SEQ-21 CDS.seq PHP32068 Wheat DGAT1-2 CDS.seq			BB1295 SEQ-21 CDS.seq PHP32068 Wheat DGAT1-2 CDS.seq			BB1295 SEQ-21 CDS.seq PHP32068 Wheat DGAT1-2 CDS.seq
113 #1		SEQ-21 58 Whea	us #1		SEQ-21 88 Whea	us #1		SEQ-21	us #1		SEQ-21	us #1		SEQ-21 8 Whea	us #1		SEQ-21	us #1		SEQ-21	us #1		SEQ-21
Consensus #1		BB1295 PHP320	Consensus #1		BB1295 PHP320	Consensus #1		BB1295 PHP320	Consensus #1		BB1295 PHP320	Consensus		BB1295 PHP320	Consensus #1		BB1295 PHP3206	Consensus		BB1295 PHP320	Consensus		BB1295 PHP320
U	850	υυ	U	- ₆ -	ს ს	4	950	4 4	H	1000	₽ ₽	۲	1050	H H	F	1100	₽ ₽	4	1150	4 A	e	1200	₽ ₽
A G		0 0 4 4	₹ ∪		4 4 U U	r U		ပပ	Ą		T E	Ţ		T T A A	D F		00	o o		ა ა ა ა	V		4 4 U U
U		υυ	U		ს ს	Ø		4 4	4		4 4	Α.		4 A	H		H H	H		H H	E		44
υ		υυ	O		ს ს	U		ს ს	æ		4 4	H		H H	ø		4 4	O		ს ს	₽		H H
<		4 4	υ		υυ	H		H H	G		0 0	H		H H	H		HH	U		ს ს	G		ט ט
Ŧ		44	υ		U U	Ā	ĺ	A A	<u>ა</u>		0 0	≮ ∪		V V	A		AA	F D		00	C F		U U
U		טט	~		A A	A	ĺ	4 4	4		4 4	4		4 4	o o		טט	4		4 4	υ		υυ
Ħ		H H	υ		υυ	H		H H	4		4 A	æ		4 4	H	_	н н	Ü	_	ს ს	U		ט ט
t	840 -	₽ ₽	H	-8-	H H	ď	940	4 4	G	990	ပ ပ	ပ	1040	υυ	E+ ·	1090	H H	U	1140	טט	H	1190	e e
E U		E E	o o		99	C L		UU	T		1 1	υ g		ပပ	o o		ဖ ဖ ဖ	A		AA	T.		T T A A
<u>«</u>		4 A	U		ບບ	H		нн	4		4 A	H		E E	E ·		H H	Ü		υυ	×		A A
ပ		ပပ	H		₽ ₽	υ		ပပ	υ		ပပ	ט		ა ა	ŋ		ა ი	4		4 4	*		A A
4		4 A	υ		ပပ	ŋ		ပ ပ	υ		ပပ	Æ		4 A	H		e e	H		H H	Ů		ს ს
ν C		V V	r U		<u>ი</u> ი	o o		0 0	Ā		A T	T.		OO	r C		OD	r C		00	0		9 9
Ü		υυ	×		4 A	F		E E	Ų		0 0	ņ		00	Α.		4 4	E		11	D T		ິດ
v		ပပ	¥		« «	K		4 4	ט		ს ს	F		H H	ပ		ပပ	Æ	١.	4 4	H		⊢ ⊢
U	830	ပပ	¥	880	⋖ ⋖	ß	930	ט ט	4	980	4 4	υ	1030	ပပ	υ	1080	ပပ	4	1130	4 4	Ø	1180	4 A
9		0 0	⋖		4 4	H		H H	٧		< <	¥	1	4 4	H	-	H H	9	~	0 0	<	1	4 4
T G		T T G	ď		0 0 4 4	C		00	o o		<u>ი</u> ი	AA		4 4 4 4	T T		11	G		5 5	ڻ د		0 G
H			H		EH EH	υ		טט	H		H H	ט		0 0	E		ΕН	υ		υυ	U		99
U		ს ს	H		ын	O		ט ט	H		₽ ₽	T		t t	₽			₽		₽ ₽	U		ს ს
H			Æ		4 4	4		4 4	¥	l	4 4	H		₽ ₽	υ		ပပ	Æ		4 4	H		e e
ب د		4 4 U U	T T		11	υ •		0 U	G A	l	0 0 V	T C		00	O.		υυ			טט	U		00
Ŀ		5 F	-			E.		H H	٠ •		4 4	r J		99	T T		TI	G		9 9	Ŧ		T A
Ð	-2-	₽ ₽	æ	870	4 A	H	920	H H	U	970	ບບ	*	1020	4 4	~	1070	4 A	U	1120	υυ	U	170	ა ა
F	ω.	₽ ₽	υ	۳	υυ	H	ľ	₽ ₽	Ü	۳.	ს ს	ט	1	ს ს	E	Ä		된	1	нн	4	117	« «
۲		< <	ڻ		טט	H		H H	Ε		H H	4		4 4	U		υυ	H		H H	U.		ა
£ 5		 	لا ن		V V	F D		n n	E G		1 T	A G		0 4 4	T		11	ı.		44	K K		A A A
H		e e	4		« «	æ		4 4	H		₽ ₽	Ü		ა ა	U		ს ს	U		טט	ų.		
Ö		ს ს	Ö		ს ს	H		⊢ ⊢	4		4 4	H			H		⊢ ←	υ		υυ	H		↔ ↔
H	!	₽₽	Ů		ს ს	ט		ს ს	¥		A A	H		₽ ₽	4		4 A	υ		υυ	H		₽₽
G		F F	U		υυ	G T		9 9	Ü		O D	T A		T T	G		9 6	C		D D	o A		0 4 4
H	810	₽ ₽	υ	860	υυ	H	-6-	H H	H	096	₽ €4	υ	10	υυ	H	1060		υ	-2-	υυ	υ	1160	υυ
ß	8	ს ს	υ	8	ပပ	¥	 	4 4	×	6	4 A	ט	101	ა ა	Ħ	ä	₽ ₽	H	111	нн	⋖	11	4 4
۴		< <	H		₽ ₽	¥		4 4	4		4 4	H		₽₽	H		₽ ₽	O		ပပ	4		4 A
N C		O O	Ţ		T A	A		A A A	T		11	GA		4 4 0 0	ე ე		ပပ ဖြ	A		A A A	A		4
A A		4 4	E		H H	-		F F	4		A A	ט		00	U		00	5		טט	ν.		00
v		υυ	ø		ს ს	¥		4 4	U		υυ	£		E+ E+	H		H H	υ		ပပ	υ		ບບ
н	'	₽ ₽	≪		4 4	F		₽ ₽	¥		4 4	H		er⊨	K		4 A	U		ပပ	O		ს ს
۲		4 4	«		N N	H		O O	F.		A T	ט		υυ	£4		9 9	0		0 0	U		00
۲		H F	υ	ı	100	υ	ı	ام م	4	ı	J~ ~	H		₽ ₽	ט	ı	la a	Ø	ı	4 A	4		4 4
		801			851 851			901			951 951			1001			1051 1051			1101			1151 1151

APPENDIX E4 WHEAT DGAT NUCLEOTIDE SEQUENCE ALIGNMENT

1201 1201

Decoration 'Decoration #1': Box residues that differ from BB1295 SEQ-21 CDS.seq.

APPENDIX F - WHEAT DGAT AMINO ACID SEQUENCE ALIGNMENT

Consensus #1 BB1295 USCWT SEQ-22.pro PHP32068 Wheat DGAT1-2.pro Consensus #1 BB1295 USCWT SEQ-22.pro PH932068 Wheat DGAT1-2.pro	Consensus #1 BB1295 USCNT SEQ-22.pro PHB32068 Wheat DGAT1-2.pro Consensus #1 BB1295 USCNT SEQ-22.pro PHB32068 Wheat DGAT1-2.pro	Consensus #1 BB1295 USCNT SEQ-22.pro PHB32068 Wheat DGAT1-2.pro Consensus #1 BB1295 USCNT SEQ-22.pro PHB32068 Wheat DGAT1-2.pro	BB1295 USCNT SEQ-22.pro PHP32068 Wheat DGAT1-2.pro Consensus #1. BB1295 USCNT SEQ-22.pro PHP32068 Wheat DGAT1-2.pro Consensus #1.	BB1295 USCNT SEQ-22.pro PHP22068 Wheat DGAT1-2.pro Consensus #1	BB1295 USCMT SEQ-22.pro PHP32068 Wheat DGAT1-2.pro Consensus #1 BB1295 USCMT SEQ-22.pro PHP32068 Wheat DGAT1-2.pro
SKGNPDPHLPGSFLPSHGGPPPKTTPPRTFRNLPSSTHGPPPGAAAA SKGNPDPHLPGSFLPSHGGPPPKTTPPRTFRNLPSSTHGPPPGAAA SKGNPDPHLPGSFLPSHGGPPFKTPPRTFRNLPSSTHGPAPSVAAA IATTPPSAAAPLPPTVHGEAAHGAAAARRDALLPGVGAAHRRVKESP	SDAIFROSHAGLLNLCIVVLIAVNSRLIIENLMKYGLLIRAGFWFSA SDAIFRQSHAGLLNLCIVVLIAVNSRLIIENLMKYGLLIRAGFWFSA GDWPLLMCCLTLPIFPLAALMTEKWAQRKLIRDHVSILLHIITTTV GDWPLLMCCLTLPIFPLAALMTEKWAQRKLIRDHVSILLHIITTTV GDWPLLMCCLTLPIFPLAALMTEKWAQRKLIRDHVSILLHIITTTTV	YP V V I I K C E S A V I S G F V I M F I A S I T W I K I V S F A H T N Y D I R I I S Q S I E K YP V V I I K C E S A V I S G F V I M F I A S I T W I K I V S F A H T N Y D I R I I S Q S I E K YP V V I I K C E S A V I S G F V I M F I A S I T W I K I V S F A H T N Y D I R I I S Q S I E K A T H G S S I D E E N I K G P T I N S V V Y F M I A P T I C Y Q P S Y P R T A F I R K G W V T R Q A T H G S S I D E E N I K G P T I N S V V Y F M I A P T I C Y Q P S Y P R T A F I R K G W V T R Q A T H G S S I D E E N I K G P T I N S V V Y F M I A P T I C Y Q P S Y P R T A F I R K G W V T R Q I K C V V F T G I M G F I I E Q Y I N P I V Q N S K H P I N G N F I D A I E R V I K I S V P T I C Y	X C V V F T G L M G F I I E Q Y I N P I V Q N S K H P L N G N F L D A I E	410 420 430 430 440 1VRHIYEPCIRNGLSKGCAILIAFLVSAVEHELCIAVPCHIFKLWAF IVRHIYEPCIRNGLSKGCAILIAFLVSAVEHELCIAVPCHIFKLWAF MFQIPLLFLTKYLQDKFKNTWVGNMIFWFFFSIVGQPMCVLYYHDV 460 450 450 55	IMFQIPLLFLTKYLODKFKNTMVGNMIFWFFFSIVGQPMCVLLYYHDVM RQAQTNG RQAQTNG RQAQTNG S'Consensus #1': When all match the residue of BB1295 USCNT SEQ-22.pro show the residue of BB1295 USCNT SEQ-22.pro,
7 7 7 HH		201 I I I I I I I I I I I I I I I I I I I	1 1 1 1	מ אא	451 G I 451 G I N R 501 N R 501 N R 501 N R 501 Consensus